

Engineered allergens for immunotherapy

Prem L. Bhalla and Mohan B. Singh

Purpose of review

Specific immunotherapy is a clinically effective causative treatment for allergic conditions. However, the reagents used for immunotherapy are crude extracts prepared from natural sources with potential life-threatening anaphylactic side effects. Molecular cloning of allergens has made it feasible to design novel therapeutic approaches for improved and safer forms of allergen-specific immunotherapy. The purpose of this review is to examine recent advances made in the last 2 years in genetic engineering of allergens for specific immunotherapy.

Recent findings

Genetic engineering of allergen with nil or low IgE reactivity but retained T-cell reactivity offers a novel therapeutic approach to improving safety and efficacy of allergen-specific immunotherapy. Hypoallergenic forms of major allergens have been produced, with reduced IgE epitopes while preserving other characteristics of the molecule to induce a protective response.

Summary

Hypoallergenic forms of major allergens are potential candidates for allergen-specific immunotherapy in the future. These genetically engineered hypoallergens now need to be tested in clinical trials before being widely used. Safer and more efficacious vaccines would increase patient compliance leading to extensive use of immunotherapy.

Keywords

genetic engineering, hypoallergens, immunotherapy, type 1 allergy

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Plant Molecular Biology and Biotechnology Laboratory, Australian Research Council Centre of Excellence for Integrative Legume Research, Institute of Land and Food Resources, The University of Melbourne, Parkville, Victoria, Australia

Correspondence to Associate Professor Prem L. Bhalla, School of Agriculture and Food Systems, The University of Melbourne, Parkville, Vic 3010, Australia
Tel/fax: +61 3 8344 9651; e-mail: premlb@unimelb.edu.au

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Introduction

Allergic diseases continue to increase in prevalence [1] and are a global health problem [2]. Conventional allergen-specific immunotherapy with allergen extracts is the only treatment that addresses the underlying cause rather than the clinical symptoms of allergy. This approach, however, suffers from the potential to cause significant side effects, including systemic and even anaphylactic responses. In addition, conventional allergen extracts contain various allergenic and nonallergic components with the potential to induce new IgE reactivities. Furthermore, variation between batches makes it very hard to standardize the concentration of the component allergens. The use of engineered proteins with reduced IgE-binding ability is now being explored as a means of providing safe and effective allergen-specific immunotherapy. Genetic engineering of allergens aims to generate modified molecules with reduced or nil IgE binding activity while maintaining structural requirements for T-cell reactivity and induction of blocking antibodies against the natural allergens. Significant progress has been made to generate hypoallergenic forms of major inhalant and contact allergens in order to improve safety and efficacy of allergen-specific immunotherapy.

Inhalant allergens

Inhalant allergens are the main cause of respiratory allergic conditions. Outdoor and indoor allergen sources have been the focus of recent studies.

Outdoor environment allergens

Pollen of grasses, weeds and trees are major sources of outdoor environment allergens. Production of hypoallergenic forms of major pollen allergens using genetic engineering is being actively investigated [3].

Engineered grass pollen allergens

Grasses of subfamily Pooideae (Tribe Poaceae) are important for agriculture. Groups 1 and 5 are the two major pollen allergens, accounting for more than 90% of IgE reactivity in most grass allergy sufferers.

Grass group 1 allergens

Grass pollen allergens belonging to group 1 are glycoproteins (28–35 kDa) [4]. These allergens are considered to be marker allergens for genuine grass pollen sensitization [5]. Comparison of protein sequences of the group 1 pollen allergens from various grass species revealed significant levels of amino acid conservation, including the presence of strictly con-

served cysteine residues, suggesting a shared protein folding pattern. There have been two approaches to modifying major group 1 allergen of ryegrass. Internal large deletions of Lol p 1 resulted in reduction in IgE reactivity [6]. However, such deleted forms may lack structures required for T-cell reactivity and induction of blocking antibodies against natural Lol p 1. The effect of conformational changes in Lol p 1 on IgE reactivity by specific replacement of the cysteine with serine residues resulted in reduction in IgE binding [7]. The modified Lol p 1 forms showed IgE reactivity that ranged from 12 to 40%, depending upon the individual patient sera tested [7]. The allergenic potential of engineered Lol p 1, however, needs to be reduced further before it can be considered as a potential candidate vaccine for grass pollen allergy. Information from a three-dimensional model of Lol p 1 [8] could be used to improve group 1 hypoallergenic forms for immunotherapy.

Grass group 5 allergens

Application of site-directed mutagenesis to replace a few key amino acids generated modified forms of r Lol p 5 that showed significant reduction in IgE reactivity [9]. It is noteworthy that these modified forms exhibited an overall structural fold similar to that of an unmodified allergen molecule [9]. The substituted amino acids were selected on the basis of their location within those IgE epitopes that showed little overlap with T-cell epitopes. The reduction in IgE reactivity of these modified forms was accompanied by a decrease in histamine release ability and skin test reactivity. In addition, these hypoallergenic forms were able to induce lymphoproliferative responses in Phl p 5-specific T-cell lines and T-cell clones. The study suggested that these Lol 5 mutants demonstrate considerable improvement over previously reported Phl p 5 mutants that needed internal deletions [9]. Further, it has been proposed that these Lol p 5 hypoallergenic forms have the potential for tenfold reduction in the occurrence of anaphylactic side effects compared with conventional immunotherapy [9]. However, further reduction in IgE reactivity of group 5 allergens by additional amino acid replacements is indeed an exciting option for better immunotherapeutic reagents in the near future.

Other grass pollen allergens

Several independent studies have identified a class of calcium binding proteins as cross-reactive allergens in the pollen of most common allergenic plants [10]. These allergens (Bra r 1, Cyn d 7, and Bet v 4) show significant IgE cross reactivities [11–14] and are considered to be responsible for polysensitization in allergic patients. The three-dimensional structure of timothy grass calcium binding allergen, Phl p 7, has been resolved [15] and disruption of the three-dimensional structure by amino acid replacements in calcium binding domains has

resulted in a hypoallergenic form [16••]. Another strategy focused on enhancing immune response by linking a less immunogenic allergen with a highly immunogenic allergen, and also linking two different allergens, has been successfully demonstrated [17]. Such a strategy of enhancing the immunogenicity could be applied to allergens from other sources.

Engineered weed pollen allergens

Parietaria, dicotyledonous weeds of family Urticaceae, commonly known as a wall pelletory, is a significant allergen source in the Mediterranean countries [18]. *Parietaria judaica* and *P. officinalis* are the most common weed species. Par j 1, a 14 kDa protein, has been identified as a major pollen allergen of *P. judaica* as it is recognized by the sera of 95% of *Parietaria*-allergic individuals [19]. Disruption of disulfide bonds by site-directed mutagenesis has led to loss of allergenicity of the variant molecules [20]. Recently, these conformational variants have been tested in a mice model system and shown to retain IgG and T-cell recognition, making them suitable candidate molecules for immunotherapy [21].

Engineered tree pollen allergens

The pollen allergens of birch, Japanese cedar and olive trees have been the subject of recent efforts towards engineering of hypoallergenic forms.

Birch pollen allergens

Birch tree pollen allergen, Bet v 1, is an important environmental allergen as 95% of birch pollen-allergic patients react with Bet v 1, and more than 60% are sensitized exclusively against Bet v 1. In addition, Bet v 1 shares B-cell and T-cell epitopes with related allergens present in the pollen of many trees and plant-derived food [22]. Various approaches used to produce hypoallergenic forms of Bet v 1 include disruption of IgE conformation epitopes by expressing Bet v 1 as two fragments and generation of a recombinant trimer. Neither of the two fragments showed IgE reactivity with sera from a number of birch pollen allergic individuals [23]. However, these fragments retained the ability to induce T-cell proliferation. In addition, the fragments were shown to have reduced anaphylactic activity as demonstrated by histamine release induced from basophils and skin prick tests. Thus a preparation containing a mixture of these fragments can be tested as a candidate for birch pollen-specific immunotherapy.

A recombinant trimer consisting of three covalently linked copies of Bet v 1 showed greatly reduced allergenic activity, but maintained similar secondary structures to wild-type Bet v 1 [24]. Bet v 1-specific B-cell and T-cell epitopes remained unaltered in the trimer form. In addition, this form induced IgG antibodies,

which blocked patients' IgE binding to Bet v 1 and related allergens. Therefore, rBet v 1 trimer was proposed as a novel hypoallergenic vaccine prototype for treatment of birch pollen allergy.

A significant milestone towards the use of the above-described engineered Bet v 1 derivatives for specific immunotherapy was achieved in a recent study reported by Mahler *et al.* [25•]. These authors tested aluminium hydroxide absorbates of two hypoallergenic rBet v 1 fragments and a hypoallergenic rBet v 1 trimer in mice. A strong IgG1 response to rBet v 1 wild-type was observed, while a weak IgE response was detected using hypoallergenic forms as immunogens [25•]. In addition, the rBet v 1 hypoallergenic forms also induced IgG1 and IgG2a/b antibodies, which cross-reacted with natural Bet v 1 and Bet v 1-related allergens from alder and hazel and blocked allergic patients' IgE binding to Bet v 1.

Recently, genetically engineered hypoallergens of Bet v 1 have been evaluated in a clinical trial involving 124 birch pollen-allergic patients [26••]. The results of this seminal study clearly showed that vaccination with the engineered allergens induced a protective response that inhibited the release of inflammatory mediators, caused reduced cutaneous sensitivity and improved clinical symptoms of the vaccinated patients. Hence the study concluded that immunotherapy with genetically engineered hypoallergens has vaccination characteristics and is a therapy that targets the underlying immunological mechanisms of allergy [26••].

Cedar pollen allergens

Japanese cedar (*Cryptomeria japonica*), commonly known as Sugi, is a forest tree widely grown in Japan. Pollen from this tree induces seasonal allergy symptoms in more than 10% of the population. The major pollen allergens, Cry j 1 and Cry j 2, have been isolated and characterized. Usui *et al.* [27] have attempted to mask IgE epitopes of Cry J 1 by chemical conjugation of the allergen molecule with galactomannan (polysaccharide). This conjugate was shown to lack IgE reactivity, leading to the suggestion that the attachment of polysaccharides to protein allergens could be used to engineer hypoallergenic forms [27].

Olive pollen allergens

Pollen from olive trees is a significant source of airborne allergens and is of high clinical relevance in the Mediterranean countries, especially Spain and Italy [28]. Ole e 1, the major allergen from olive pollen, has been well characterized in terms of its structural (sequence, glycosylation degree, pI, etc.) and immunological features [29]. Comparison of the IgE reactivities of two isoforms of Ole e 1 expressed as recombinant proteins [30] showed that one of the isoforms, rOle e 1/

5c, displayed reduced IgE binding. However, further reduction in IgE reactivity is required for its potential use as a hypoallergen for immunotherapy.

Indoor environment allergens

House dust mite allergens and cat dander allergens are the major indoor elicitors of allergic disorders. Allergens from the house dust mites are ubiquitous in house dust. Exposure to these allergens can cause acute symptoms in patients who are sensitized to these allergens and have asthma or other allergic diseases, such as rhinitis or atopic dermatitis.

Engineered house dust mite allergens

Group 1 and 2 allergens are the major allergens of house dust mite [31]. Site-directed mutagenesis, expression of polypeptide fragments and introduction of single amino acid approaches have been used to generate hypoallergenic forms. Modified Der p 2 forms carrying substitutions at cysteine residues resulted in a more than 100-fold reduction in IgE reactivity and a significant reduction in cutaneous sensitivity [32]. A hypoallergenic variant of Der f 2 has been generated by removal of the disulfide bond between the N and C-terminal sequences [33]. Substitution of the proline residues in Der f 2 also reduces the allergenicity [34]. Expression of rDer f 2 as two separate (1–112 amino acids, and 85–129 amino acids) polypeptides resulted in loss of IgE reactivity and cutaneous sensitivity [35]. Thus these variants could offer a safer and more effective candidate vaccine for house dust mite allergy.

Engineered cat dander allergens

The dander from the domestic cat (*Felis domesticus*) is one of the most important elicitors of allergic asthma worldwide. The major cat allergen, Fel d 1, a 35–39 kDa protein, is composed of two heterodimers. Nearly 90–95% of cat-allergic patients show IgE reactivity to Fel d 1 [36].

Immunotherapy with a T-cell epitope containing Fel d 1 synthetic peptides showed the occurrence of undesirable systemic side effects [37]. The development of hypoallergenic forms of Fel d 1 has been hampered by difficulties in expression of the recombinant molecule in heterologous host systems. An attempt to express properly folded recombinant Fel d 1 has been successful recently [38,39]. The recombinant Fel d 1, consisting of two chains fused together, has been shown to possess immunological properties indistinguishable from the natural protein molecule. In addition, the three-dimensional structure of Fel d 1 has also been recently discovered [38], providing a valuable tool towards designing and producing hypoallergenic forms that can be tested as potential candidates for cat dander immunotherapy.

Table 1. Allergens from various sources engineered for reduced anaphylactic potential

Allergen	Allergen source	Approach	IgE reactivity	T-cell reactivity	Skin prick reactivity	Reference
Inhalant allergens						
Outdoor environment allergens						
Grass pollen						
Lol p 1	Ryegrass	Internal deletions	Low	ND	ND	Tamborini <i>et al.</i> [6]
Lol p 1	Ryegrass	Disulfide bond variants	Low	ND	ND	de Weerd <i>et al.</i> [7]
Lol p 5	Ryegrass	Amino acid replacements	Low	Retained	Reduced	Swoboda <i>et al.</i> [9]
Phl p 5	Timothy grass	Internal deletions	Low	Retained	Reduced	Schramm <i>et al.</i> [44]
Phl p 7	Timothy grass	Synthetic fragments	Abolished	ND	Abolished	Westritschnig <i>et al.</i> [16••]
Tree pollen						
Bet v 1	Birch	Break in two fragments	Nil to low	Retained	Low to nil	Vrtala <i>et al.</i> [23]
Bet v 1	Birch	Amino acid replacements	Low	Retained	ND	Vrtala <i>et al.</i> [23]
Bet v 1	Birch	Trimer formation	Low	Retained	Low to nil	Vrtala <i>et al.</i> [24]
Bet v 4	Birch	Site-directed mutagenesis	No change	ND	ND	Engel <i>et al.</i> [13]
Cry j 1	Cedar	Polysaccharide attachment	Abolished	ND	ND	Usui <i>et al.</i> 2003 [27]
Ole e 1	Olive	Hypoallergenic isoform	Low	ND	ND	Gonzalez <i>et al.</i> [30]
Weed pollen						
Par j 1	<i>Parietaria</i>	Disulfide bond variants	Low to nil	Retained	Low to nil	Bonura <i>et al.</i> [20] Orlandi <i>et al.</i> [21]
Indoor environment allergens						
Der p 2	House dust mite	Disulfide bond variants	Reduced	ND	Reduced	Smith and Chapman [32] Takai <i>et al.</i> [33]
Der f 2	House dust mite	Amino acid replacements	Reduced	ND	Abolished	
Contact allergens						
Hev b 6.02	Rubber latex	Amino acid replacements	Reduced	Retained	Abolished	Karisola <i>et al.</i> [42••]

ND, not determined.

Contact allergens: engineered natural rubber latex allergens

Natural rubber latex allergy is well known, especially among health care workers and children with spina bifida. The proteins that elute from the surface of latex products are responsible for type I hypersensitivity. Hevein (Hev b 6.02), a small (4.7 kDa; 43 amino acids) protein, is one of the most important natural rubber latex allergens, with 70% of allergic patients showing IgE reactivity and skin prick sensitivity [40,41].

Recently, Karisola *et al.* [42••] reported on the production of hypoallergenic forms of hevein by mutating six amino acids in the conformational epitope. The conformational IgE-reactive epitopes were identified by the use of an antimicrobial protein, AMP, from amaranth, a non-IgE-reactive orthologue of hevein. The strategy used to map IgE epitopes included use of this antimicrobial protein as an adapter to make chimeric molecules that carried different small segments of hevein. The study led to the conclusion that major IgE-binding epitopes of hevein are conformational and these epitopes are localized in its N and C-terminal regions [43]. In addition to a dramatic reduction in IgE-binding capacity of the mutants, skin prick reactivity with the six-residue mutant, H 6, was completely abolished. Thus this engineered hevein form may be a valuable candidate for immunotherapy of latex allergy.

Conclusion

Engineering of wild-type allergens using recombinant techniques with reduced allergenic activity (Table 1) has

emerged as the most promising strategy for the development of improved allergy vaccines. The application of modern bioinformatics tools [45] and elucidation of the three-dimensional structure of major allergens are poised to provide great impetus to further develop recombinant derivatives with greatly reduced anaphylactic potential [46]. In addition, a three-dimensional structure will also facilitate better prediction of cross reactivities [47] and should allow the development of immunotherapy vaccines able to induce protective responses against a wide range of cross-reactive allergens. Hence, new therapeutic agents with enhanced safety and efficacy will be based on developing hypoallergenic forms via genetic engineering.

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